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Relative impacts of morphological alteration to shorelines and eutrophication on littoral macroinvertebrates in Mediterranean lakes

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Abstract: Development of effective methods for assessing the ecological status of lakes based on littoral benthic fauna has been hampered by the lack of quantitative data on the relative impacts of key pressures on the benthic community. We used variance partitioning at 126 sites belonging to 14 natural Mediterranean lakes to analyze the pure and shared effects of eutrophication, morphological alterations, microhabitat type, lake morphometry and geographic position on the littoral macroinvertebrate community. The spatial arrangement of the sampling sites was responsible for 9.1% of the total variance in littoral benthic community composition, lake morphometry accounted for 4.3% of variation, and microhabitat type accounted for 3.9%. Communities appeared to be affected primarily by morphological alterations to lake shorelines, and their impact was 2.5 times as important as that of eutrophication. The structure of littoral benthic communities was governed by processes acting at several spatial scales from region to lake scale. Thus, several pressures and the various spatial scales at which these act should be taken into account when implementing methods of assessing lake ecological condition based on littoral benthic invertebrates. Region-specific methods for subalpine and volcanic lakes might enhance the validity of assessment of results of morphological alterations and improve management of those water resources.
Key words: lake, littoral zone, invertebrate community, morphological alteration, eutrophication, microhabitat, variance partitioning, spatial analysis, Water Framework Directive, multiple pressures

Freshwater ecosystems experience multiple stressors acting simultaneously (Ormerod et al. 2010). The effects of one stressor can potentially modify the effects of others through additive, antagonistic, or synergistic interactions (Darling and Côté 2008). A major challenge when assessing and managing the ecological quality of water bodies is to quantify the single and combined effects of multiple stressors on the biota (Solimini et al. 2009).

Lakes are exposed to several human stressors including eutrophication, acidification, modification of lake water level, and shoreline morphological alterations (Rasmussen and Kalff 1987, Skjelkvåle et al. 2001, Brauns et al. 2007b, Mastrantuono et al. 2008) that act over several spatial and temporal scales (Hämäläinen et al. 2003, Solimini et al. 2003, Stoffels et al. 2005). Invertebrate assemblages of the littoral, sublittoral, and profundal zone may respond differently to human pressures (Hutchinson 1993). For example, alteration of species composition of the profundal invertebrate assemblage in response to eutrophication is well known (Jonasson 1972), whereas sublittoral and littoral assemblages do not show clear response patterns (Brauns et al. 2007b, Bazzanti et al. 2012, McGoff and Sandin 2012). Community composition of lake littoral invertebrates is affected by acidification (Schartau et al. 2008, Wesolek et al. 2010) and by morphological alteration of lakeshores (Solimini and Sandin 2012, Miler et al.

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2013, McGoff et al. 2013a). In the European Mediterranean region, the current lack of comparative data on the relative impacts of various pressures on benthic assemblages inhabiting different lake zones hinders the development and application of assessment methods (Birk et al. 2012), which are needed to implement legal requirements of the Water Framework Directive (EC 2000).

The littoral zone of lakes hosts higher species richness of invertebrates than sublittoral and profundal zones because of higher habitat diversity (White and Irvine 2003). Therefore, the invertebrate assemblages of this lake zone are potentially powerful indicators of anthropogenic pressures. Within this context, the intensity of their responses to specific pressures must be disentangled and demonstrated in a quantitative way. Current knowledge predicts that benthic communities in the littoral zone of lakes are mainly affected by morphological alteration and acidification, whereas eutrophication has a minor effect (see recent review by Solimini and Sandin 2012). Hydromorphological alterations, which encompass alteration of the hydrological regime and morphological alteration of the shoreline, can decrease invertebrate species diversity and abundance (Bänziger 1995), alter the taxonomic and functional structure (Brauns et al. 2007b), reduce richness of plant-associated sessile invertebrates (Mastrantuono et al. 2008), and affect littoral habitat quality (McGoff and Irvine 2009). The effect of eutrophication was addressed explicitly by Donohue et al. (2009), who developed an ecological classification model based on changes of littoral invertebrate assemblages across a gradient of nutrient enrichment. However, the strength of the response of eulittoral invertebrates to different trophic states among lakes depends on the habitats sampled (Brauns et al. 2007b).

Previous work on littoral zones of lakes was mainly focused on the effects of single pressures (Brauns et al. 2007a, b, Mastrantuono et al. 2008, O'Toole et al. 2008). The relative impacts of multiple stressors and their combined effects remain largely unknown. We investigated the community composition of littoral macroinvertebrate assemblages of Italian lakes in 2 regions and their relationships with several environmental variables. We chose these regions because they belong to the Mediterranean ecoregion, but differ in natural condition (i.e., geology and lake morphology) and levels of anthropogenic pressures, which may result in different abiotic–biotic associations. Our primary aim was to quantify the relative effects of morphological alteration and eutrophication on the benthic invertebrate community in the littoral zone of natural Mediterranean lakes, once we had accounted for other environmental gradients and spatial variation. Moreover, we wanted to identify indicator taxa associated with environmental gradients, microhabitat types, and varying levels of anthropogenic pressures on these lake ecosystems. We hypothesized that littoral benthic communities are more strongly affected by morphological alteration than eutrophication.

METHODS

Study area

We analyzed littoral invertebrates in 14 natural lakes in northern (n = 8) and central Italy (n = 6). These lakes were studied as part of a large pan-European collaborative project (EU-project WISER; Hering et al. 2010) and are affected by a wide range of morphological and eutrophication pressures (Fig. 1). All lakes belong to ecoregion 3 of the European Ecoregion Classification System of lakes and rivers (EC 2000) and vary in size and depth (Table 1). Our selection of lakes was based on the analysis of the land use neighboring the shoreline, trophic level, and geographic characteristics obtained from available geographic information system (GIS) maps, Google Earth® images, and historical water-quality data.

The sampled lakes in northern Italy are of glacial origin. Scouring by glaciers during the Quaternary contributed to the formation of depressions, which subsequently filled with water. In contrast, the lakes in central Italy are of volcanic origin. They were formed as a result of Quaternary volcanism that created the craters and calderas that today host those basins. Volcanic lakes are generally circular and much deeper than glacial lakes.

We selected 3 unmodified sites, 3 sites with 'soft' anthropogenic modifications, and 3 with 'hard' anthropogenic modifications in each lake (total: 126 sites). Unmodified sites were defined as those sites not affected by human-induced morphological alterations, with natural or semi-natural fringing and littoral vegetation. 'Soft' altered sites were defined as sites affected by the removal of fringing and littoral vegetation and by the presence of artificial recreational beaches. 'Hard' altered sites were those sites affected by hard engineering structures (i.e., concrete walls, rip-rap, gabions, and others). The sites were 25 m wide (parallel to

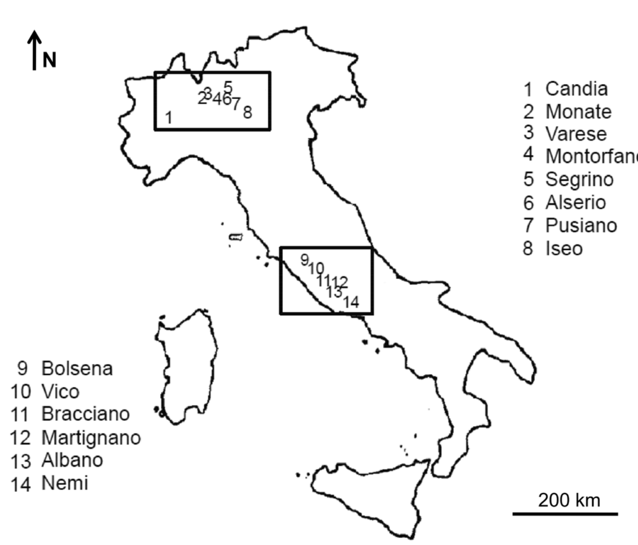

Figure 1. Map of Italy showing the 14 study lakes.

Table 1. Morphometric characteristics of the studied lakes in central and north Italy.

Region	Lake	Latitude (°N)	Longitude (°E)	Surface (km^2)	Volume ($\times 10^6$ m^3)	Maximum depth (m)	Mean depth (m)
Central Italy	Albano	41.747	12.671	6.02	464.2	170	77
	Bolsena	42.596	11.945	114.53	8922.0	146	81
	Bracciano	42.122	12.236	57.50	4950.0	160	89
	Martignano	42.126	12.233	2.50	71.2	54	43
	Nemi	41.713	12.704	1.67	32.5	34	17
	Vico	42.317	12.175	12.10	268.0	49	23
Northern Italy	Alserio	45.787	9.215	1.23	6.6	8	5
	Candia	45.325	7.912	1.35	8.1	8	5
	Iseo	45.737	10.072	61.00	7600.0	251	124
	Monate	45.795	8.665	2.50	45.0	34	18
	Montorfano	45.783	9.138	0.46	1.9	7	4
	Pusiano	45.804	9.274	4.95	69.2	24	14
	Segrino	45.830	9.268	0.38	1.2	9	4
	Varese	45.811	8.745	14.56	100.0	26	11

the shoreline) and were sampled until the greatest wadeable depth.

Environmental variables

We conducted the morphological assessment by applying the Lake Habitat Survey (LHS) protocol (Rowan et al. 2006). The LHS protocol consists of surveys in 10 areas (habplots) uniformly spaced along the perimeter of the lake. In each habplot, we recorded on field sheets provided by the protocol the features of the riparian zone (defined as the zone extending 15 m landward from the bank edge), the shore, and the littoral zone (defined as the zone extending 10 m offshore from the water line), and the presence of anthropogenic pressures (e.g., commercial and residential activity, roads) within a 50-m radius from the habplot. The features occurring between 2 adjacent habplots were recorded as part of the whole-lake assessment (Rowan et al. 2006). We calculated 2 indices from the LHS data to synthesize morphological conditions at whole-lake scale: the lake habitat quality assessment (LHQA-total) and the lake habitat modification score (LHMS). The LHQA score summarizes the diversity and the quality of lake habitats. The LHMS scores the overall anthropogenic pressures occurring at whole-lake scale. High values of the LHMS index indicate higher anthropogenic pressures at whole-lake scale. We also included in our analysis 3 partial scores of the LHQA computation protocol, which are indices of the morphological quality of the riparian, shore, and littoral zones (LHQA-riparian, LHQA-shore, and LHQA-littoral) at whole-lake scale.

In addition, at each sampling site, we computed 2 indices of morphological conditions at site scale: the Habplot Quality Assessment (HabQA) score and the total pressures index. The HabQA score is analogous to the LHQA in that it is an indicator of quality and diversity of habitats, but it differs from the LHQA because it assigns a score to each site within a lake (McGoff and Irvine 2009). The total pressures index is analogous to the LHMS, but it assigns a score to each individual site. It represents the occurrence of 18 potential anthropogenic pressures affecting the riparian zone and the shoreline within a 50-m radius of each site (Free et al. 2009).

We used aerial photographs from Google Earth to estimate the land use within a 200-m zone from the lake edge. We defined 3 classes of land use: natural coverage (which included forested areas, shrubs and scrubs, and wetlands), urban, and agricultural. We used land use in proximity to the lakeshores as an indicator of morphological alterations (following Rowan et al. 2006, McGoff et al. 2013b). For the image analysis, we used the software ImageJ (US National Institutes of Health, Bethesda, Maryland).

We obtained physicochemical variables at the whole-lake scale (total P [TP], total N [TN], chlorophyll *a*, and Secchi depth) from national water-quality databases (www.sintai.sinanet.apat.it). We used the averages of values measured along the water column at mid-lake over a 12-mo period of the latest available year in the database.

We measured site-level physicochemical and morphological variables at the time of invertebrate sampling. We measured dissolved O_2, temperature, pH, and conductivity with a multiparameter probe (Multi 340i; WTW, Weilheim, Germany) at the deepest point at each site. We estimated the slope of the littoral zone from the water depth at a distance of 5 m from the shoreline. We collected a 100-mL water sample at each site and measured P and N concentrations with standard spectrophotometric methods as described by Valderrama (1981). We also filtered 500 mL of water for chlorophyll *a* analysis. We removed periphyton from a 3 × 3-cm surface area of a rock or submersed hard substrate using a small brush and quantified it as chlorophyll *a* density. We measured chlorophyll *a* following Standard Method 10200 H (APHA 1995). All chemical analyses were carried out by the Chemical Monitoring laboratory, Institute for Environment and Sustainability, Joint Research Centre (Ispra, Italy).

Macroinvertebrate sampling

We collected macroinvertebrates in late summer 2009. At each sampling site, we collected 3 samples from the 3 most abundant microhabitats (visually assessed) with a 500-μm-mesh hand net. We collected each sample from 1 m^2 of surface area delimited with a plastic quadrat placed on the lake bottom. We grouped microhabitat types into 3 classes: stones (boulders, cobbles, rocks, and artificial hard substrates, such as concrete walls and rip-raps), sand (fine sediments from gravel to silt), and macrophytes (reed stems and submerged macrophytes). We used kick samples in fine-grained substrates (silt, sand, and gravel). We collected cobbles and boulders and thoroughly rinsed them in the net to remove all the organisms. We scraped in situ rocks or man-made features, such as walls, to collect the epibenthic organisms. We rinsed submerged and floating macrophytes in the net and scraped helophyte stems (*Phragmites* sp., *Typha* sp., Cyperaceae, *Juncus* sp.) with the hand net.

We stored samples in the field in plastic jars with 90% ethanol and transported them to the laboratory for processing. In the laboratory, we split each sample into 2 parts with a Södergren subsampler (Södergren 1974) and processed 1 part by washing it through nested sieves (mesh sizes: 5.0 mm, 1.0 mm, and 0.5 mm). We placed a portion of the largest size fractions (>5 mm and >1 mm) in a white tray, diluted it with water as needed, and sorted the sample under 4× magnification for macroinvertebrates, which we preserved in 70% ethanol for later identification. We checked the remaining material under 25× magnification before discarding. We repeated this procedure until ½ of the sample had been completely sorted. We diluted the material retained by 0.5-mm sieves with 500 mL water and collected three 50-mL subsamples with a syringe. We sorted the subsamples under 25× magnification. We identified specimens to species or genus, except for Oligochaeta (class) and Diptera (family). We calculated invertebrate abundances at the site level as averages of the microhabitat-specific abundances weighted by the % cover of each sampled microhabitat.

Statistical analyses

Prior to the statistical analyses, invertebrate abundances were Hellinger-transformed. The Hellinger transformation, when associated with Euclidean-based ordination methods, such as redundancy analysis (RDA), preserves the Hellinger distance among sites, which down-weights the most abundant taxa (Legendre and Gallagher 2001). Moreover, in contrast to canonical correspondence analysis (CCA), RDA applied to Hellinger-transformed data does not overweight rare taxa (Legendre and Gallagher 2001).

Identification of environmental gradients We used 26 environmental variables organized in 5 groups: morphological alteration (10), eutrophication (9), microhabitat (3), morphometry and alkalinity (3), and slope of the littoral zone (Table 2). The variables related to morphological alterations and eutrophication were further divided into pressures acting at the site and lake levels to account for the effects of those pressures at different spatial scales. We ran a principal components analysis (PCA) on each group of variables to identify major environmental gradients, except for the slope of the littoral zone, which represented a gradient per se. We selected enough principal components (PCs) for inclusion in subsequent analyses to represent >50% of the cumulative variance of the given environmental matrix. These PCs were: PC1 for morphological alteration at site level, eutrophication at site and lake levels, morphometry and alkalinity, and PCs 1 and 2 for morphological alteration at the lake level (Table 3).

Multivariate analyses of assemblage composition We examined the relationship between invertebrate community composition and gradients of environmental variables and pressures with constrained ordinations of assemblage composition data based on redundancy analysis (RDA) as implemented in the R package *vegan* (version 2.0–8; R Project for Statistical Computing, Vienna, Austria). RDA ordination is based on computation of orthogonal axes that are linear combinations of explanatory variables that best explain the variation of the assemblage composition (Borcard et al. 2011). Thus, this method enables comparison of the strength of the statistical association of each explanatory variable with the biotic community. The analysis can be done as a partial (or conditional) RDA to account for the influences of nuisance variables or as variance-partitioning analysis to decompose the variance of a response matrix (biological data) among ≥2 groups of explanatory variables to identify their unique and shared contributions to total variance (Borcard et al. 1992, Legendre and Legendre 2012).

For the 1st approach, we ran a partial RDA with the microhabitat types and the environmental gradients (identi-

Table 2. Spatial scale and ranges of environmental variables. HabQA = Habplot Quality Assessment, LHMS = lake habitat modification score, LHQA = lake habitat quality assessment, TP = total P, TN = total N, Chl*a* = chlorophyll *a*.

Variable group	Spatial scale	Variable	Range
Morphological alteration	Site	HabQA	2.50–9.25
	Site	Total pressures index	0–9
	Lake	LHMS	14–30
	Lake	LHQA-total	37–66
	Lake	LHQA-riparian	5–11
	Lake	LHQA-shore	6–17
	Lake	LHQA-littoral	12–26
	Lake	Landuse natural (%)	36.6–96.6
	Lake	Landuse agriculture (%)	0–56.5
	Lake	Landuse urban (%)	1.4–61.7
Eutrophication pressure	Site	O_2 (%)	76.4–226.0
	Site	Phytoplankton biomass (μg Chl*a*/L)	0.6–45.3
	Site	Periphyton biomass (μg Chl*a*/cm^2)	0.2–31.9
	Site	TP (μg/L)	0.2–111.6
	Site	TN (mg/L)	0.10–1.54
	Lake	TN mid-lake (mg/L)	0.05–1.80
	Lake	TP mid-lake (μg/L)	8–130
	Lake	Phytoplankton biomass mid lake (μg Chl*a*/L)	0.6–22.0
	Lake	Secchi depth mid lake (m)	1.5–10.0
Microhabitat type	Site	Sand (%)	0–100
	Site	Macrophyte (%)	0–100
	Site	Stone (%)	0–100
Morphometry and alkalinity	Lake	Surface area (km^2)	0.38–114.53
	Lake	Maximum depth (m)	7–251
	Lake	Alkalinity mid lake (meq/L)	0.83–4.65
Slope of the littoral zone	Site	Depth 5 m from the shore (m)	0.15–45.00

fied by the principal components of the PCAs as described above) as explanatory variables. We were interested in the effects of environmental gradients in structuring the invertebrate community, so we partialled out the regional effect by conditioning the analysis by region, which was coded as a dummy variable.

For the 2nd approach, we ran the variance-partitioning analysis on the following groups of explanatory variables: 1) spatial component (see below), 2) microhabitat types, 3) morphometry/alkalinity (PC1 and slope of the littoral zone), 4) morphological alterations (PC1 at site level, PC1 and PC2 at lake level), 5) eutrophication (PC1 at site and lake level). We conducted variance partitioning with the *varpart* function in *vegan*, which computes adjusted R^2 values. The adjustment allows a comparison of models with differing numbers of predictors and sample sizes (Peres-Neto et al. 2006). *varpart* allows partitioning of variance among a maximum of 4 groups of variables, whereas our study design included 5 groups. Aggregation of 2 groups of variables did not affect the estimation of the unique effects of the remaining groups, so we ran 2 variance-partitioning analyses with different aggregations of the groups. In the 1st run, we aggregated the spatial components and morphometry/alkalinity groups, and in the 2nd run, we aggregated eutrophication and morphological alteration. In this way, we could quantify the unique effect of each of the 5 groups of variables.

Spatial analysis The spatial structure of biotic communities is the result of various processes, such as biotic interactions, dispersal, and processes related to spatially structured environmental factors (Borcard and Legendre 1994). Thus, we quantitatively described the spatial pattern of our study design and used variance partitioning to assess its contribution to community variance and, subsequently, to distinguish its effects from those resulting from environmental factors and anthropogenic pressures (Borcard et al. 1992, Legendre and Legendre 2012).

We coded the 2 regions (northern and central Italy) as a dummy binary variable (Region) and assessed regional

Table 3. Results of principal components analysis (PCA). A PCA was run for each group of environmental variables. For morphological alteration and eutrophication, 2 different PCAs were run. One included variables assessed at the site level and the other included variables assessed at lake level. The variance explained by the first 2 principal components (PCs) of each PCA is reported.

Variable group	Spatial scale	Variance explained	
		PC1	PC2
Morphological alteration	Site	0.71	0.29
	Lake	0.37	0.25
Eutrophication pressure	Site	0.53	0.19
	Lake	0.60	0.21
Morphometry and alkalinity		0.62	0.28

differences in the littoral benthic community with RDA. We tested the model for significance with a permutation test (Borcard et al. 2011). We quantitatively described the spatial structure of the sampling design via a global, hierarchical spatial analysis using Moran's eigenvector maps (MEM; Borcard et al. 2004, Dray et al. 2006, Declerck et al. 2011). We computed MEMs from the geographic coordinates of the sampling sites. They were obtained by principal coordinate analysis (PCoA) of a truncated matrix of distances among the sampling sites. To account for large (regional) and fine (between and within lakes) spatial scales, we computed MEMs independently for northern and central Italy and assembled them in a staggered matrix as described in Declerck et al. (2011). We used the function create.MEM.model() provided in appendix 1 by Declerck et al. (2011).

We selected significant MEMs for each region with a forward-selection procedure. This procedure uses the results of a Monte Carlo test with 999 random permutations to test the significance of explanatory variables successively entering the model (Léonard et al. 2007, Brind'Amour et al. 2009). In addition to the usual α significance level (<0.05) we applied a 2nd stopping criterion to reduce Type 1 errors (Blanchet et al. 2008). The 2nd stopping criterion terminated the forward-selection procedure if the R^2 of the model exceeded the adjusted R^2 of the full model, which included all the variables (Blanchet et al. 2008). We ran this analysis using the R package *Packfor* (version 0.0–8).

Indicator taxa of morphological alterations of the shoreline We tested for differences in the invertebrate community composition among levels of the anthropogenic morphological alteration factor (unmodified, soft, and hard altered shorelines) with a partial RDA with the nested effects of region and lake partialled out of the analysis. We tested the model for significance with a permutation test (Borcard et al. 2011).

We computed the indicator value index (IndVal; Dufrêne and Legendre 1997, De Cáceres and Legendre 2009) to identify indicator taxa of each level. IndVal enables identification of indicator taxa for a specific group of sites. A taxon is an indicator for a certain group of sites if it has a large mean abundance within that group compared to the other groups and if it is present in most sites of that group (Borcard et al. 2011). We obtained the significance of each indicator value by a permutation test. We ran this analysis in the R package *indicspecies* (version 1.6.5).

RESULTS

Our data set included 167,011 invertebrates from 161 taxa. The most abundant taxa were Oligochaeta (18,469 individuals [ind]) and Chironomidae (14,099 ind). The taxonomic groups with higher numbers of taxa were Odonata (29), Gastropoda (24), and Trichoptera (23).

Spatial analysis

The effect of Region was significant in shaping the benthic community composition (permutation test for RDA, $F_1 = 14.93$, $p < 0.01$) and explained 11.1% of the variance in macroinvertebrate assemblage composition. The spatial analysis led to the identification of 8 MEMs, which discriminated among lakes and among groups of neighboring lakes within each region. The forward-selection procedure selected 5 of those MEMs, 3 for central Italy and 2 for northern Italy, which were included in subsequent variance-partitioning analysis as spatial descriptors, together with Region (coded as dummy variable).

Identification of environmental gradients

The PCs resulting from the different PCAs identified the major environmental gradients (Tables 3, 4). Increasing scores along PC1 for site-level morphological alterations indicated declining habitat quality and increasing anthropogenic pressures in the surrounding area, whereas increasing scores along PC1 for lake-level morphological alterations indicated declining habitat quality and increasing agricultural land use. Increasing scores along PC2 for lake-level morphological alterations represent increasing modification to lake shorelines associated with urban land use. Scores along PC1 for site-level eutrophication increased with increasing values of periphyton biomass, TN, and TP, whereas scores along PC1 for lake-level eutrophication increased with increasing values of phytoplankton biomass, TN, and TP, and decreasing values of Secchi depth. Scores along PC1 for lake-level morphomet-

Table 4. Results of principal components analysis (PCA). A PCA was run for each group of environmental variables. For morphological alteration and eutrophication, 2 different PCAs were run. One included variables assessed at the site level and the other included variables assessed at lake level. The table shows the loadings of the principal components (PCs) that overall represent >50% of the cumulative variance of the environmental matrix. HabQA = Habplot Quality Assessment, LHMS = lake habitat modification score, LHQA = habitat quality assessment, TP = total P, TN = total N.

Variable group	Spatial scale	Variable	Loading on PC1	Loading on PC2
Morphological alteration	Site	HabQA	−0.71	
		Total pressures index	0.71	
	Lake	LHQA-total	−0.49	0.21
		LHMS	0	0.44
		LHQA-shore	−0.24	−0.17
		LHQA-littoral	−0.42	0
		LHQA-riparian	−0.49	−0.23
		Landuse urban	−0.28	0.53
		Landuse agriculture	0.42	0.12
		Landuse natural	−0.13	−0.23
Eutrophication pressure	Site	O_2	0.32	
		Phytoplankton biomass	0.58	
		TP	0.46	
		TN	0.56	
		Periphyton biomass	0.21	
	Lake	TP mid lake	0.51	
		TN mid lake	0.55	
		Secchi depth mid lake	−0.31	
		Phytoplankton biomass mid lake	0.59	
Morphometry and alkalinity		Surface area	0.65	
		Maximum depth	0.65	
		Alkalinity mid lake	0.40	

ric variables and alkalinity increased with increasing maximum depth and lake surface area.

Partial RDA and indicator taxa of morphological alterations of the shoreline

Environmental gradients explained 21.1% (adjusted R^2) of the variance in invertebrate composition after partialling out the spatial effect of Region (11.1% of total variance). The 1st RDA axis (RD1; explained 4.1% of variance) was positively correlated with sand microhabitat and negatively correlated with the lake size, lake-scale morphological alteration (both PCA components), and stone microhabitat. RD2 (explained 1.8% of variance) was positively correlated to sand microhabitat and morphological alterations at site level and negatively correlated to macrophyte microhabitat.

Several taxa were strongly associated with RDA gradients (Fig. 2). In particular, Oligochaeta were associated with sandy substrates, Ceratopogonidae and *Dugesia* sp. with increasing site-level eutrophication, *Caenis macrura* group and *Dreissena polymorpha* with stones, altered shorelines and lake size, and *Echinogammarus veneris* with increasing lake size. *Ischnura elegans* and *Ecnomus tenellus* were associated with macrophytes, and *Bithynia tentaculata* were associated with macrophytes and stones.

The invertebrate community structure differed among levels of the anthropogenic morphological alteration (permutation test for partial RDA, $p < 0.01$). Indicator species analysis showed that 8 taxa were indicators of unmodified sites, 3 were indicators of soft altered sites, and 13 were indicators of hard altered sites (Table 5).

Variance partitioning

The selected variables explained 31.9% of total taxa variance. The main explanatory group was the spatial pattern among the sampling sites, which accounted for 9.1% of total variance ($p < 0.01$; Fig. 3, Table S1). The unique effect of morphological alterations accounted for 2.0% ($p < 0.01$) of total variance, and eutrophication accounted for 0.8% ($p < 0.05$). The shared variation between morphological alterations and eutrophication accounted for 1.3% of

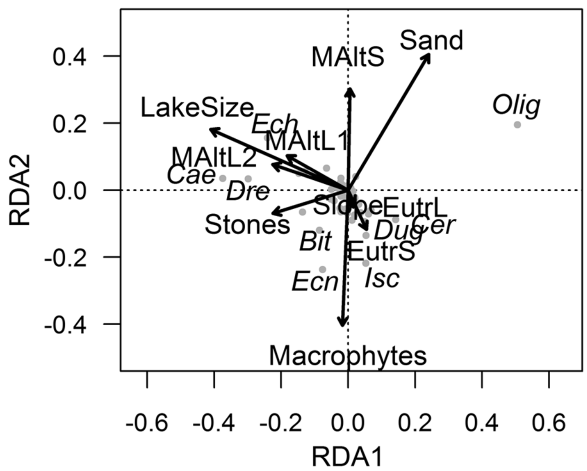

Figure 2. Biplot of species and site scores obtained from a partial redundancy analysis (RDA) of environmental variables and indicators of anthropogenic impacts. The effects of region and lakes nested within region were partialled out (see text). Arrows show the strength and direction of maximum correlation for variables representing eutrophication, morphological alteration, morphometric variables and microhabitat. Biplot is scaled by species scores. Grey dots = sampling sites; black dots = taxa (names are displayed only for taxa correlated with environmental variable vectors). MAltS = morphological alteration at site level (1st principal component); MAltL1 = morphological alteration at lake level (1st principal component); MAltL2 = morphological alteration at lake level (2nd principal component); EutrS = eutrophication at site level (1st principal component); EutrL = eutrophication at lake level (1st principal component); LakeSize = 1st principal component of the morphometric variables, Slope = depth at 5 m from the shore; Sand, Stones, and Macrophytes = percentages of the microhabitats sand, stone, and macrophytes; Bit = *Bithynia tentaculata*, Cae = *Caenis macrura*, Cer = Ceratopogonidae, Dre = *Dreissena polymorpha*, Dug = *Dugesia* sp., Ech = *Echinogammarus veneris*, Ecn = *Ecnomus tenellus*, Isc = *Ischnura elegans*, Olig = Oligochaeta.

total variance. The shared variation between the spatial descriptors and morphological alterations accounted for 4.1% of total variance, and the shared variation between the spatial descriptors and eutrophication accounted for 3.8% of total variance.

DISCUSSION

Our results suggest that the spatial pattern among the sampling sites was responsible for a large part of the total variance of littoral benthic communities. Littoral macroinvertebrate communities were more strongly associated with morphological alterations than with eutrophication, a result suggesting a stronger impact of morphological alterations than of eutrophication on the littoral benthic communities.

Spatial and environmental variables and whole community variance

Spatial variables were responsible for a large part of the benthic-invertebrate community variance across the study region. Region was statistically significant in the RDA, indicating the importance of the regional component on the structure of invertebrate community. These large-scale differences may be the result of regional differences in environmental variables, such as climate or catchment characteristics. The 2 regional groups of lakes have different geological origin (glacial and volcanic), which influences the catchment characteristics and the lake morphology. An alternative explanation is that the large-scale differences may be a consequence of purely spatial phenomena, such as limitations on dispersal. The importance of geographical and morphological characteristics in explaining invertebrate community variation supports the typological approach used in the ecological assessment in the implementation of the Water Framework Directive (Nõges et al. 2009). This implementation consists of a type-specific assessment of the ecological status of water bodies, where typologies are defined on the basis of the geographic, geological, and morphological characteristics of the catchment and water body (e.g., altitude, geology, depth, and size). Our study design did not allow description of within-lakes spatial structures. However, the spatial component (MEMs vectors) accounted for among-lakes differences, so we can infer that the proportion of the community variance that was not explained by our model (i.e., the residuals of the variance-partitioning analysis: 68.1%) probably represents within-lakes variance. Microhabitat types (i.e., the smallest

Table 5. Indicator taxa of morphologically altered shorelines. The significance of the indicator value is shown: * = $p \leq 0.05$, ** $p \leq 0.01$.

Indicator taxa	
Unmodified sites	Hard altered sites
*Ischnura elegans***	Chironomidae**
Limoniidae**	*Ecnomus tenellus***
*Echinogammarus veneris**	*Hydroptila* sp.**
*Palaemonetes antennarius**	*Ceratopogonidae***
Cloeon simile-Gr.*	*Theodoxus fluviatilis***
*Orthotrichia costalis**	*Unio* sp.*
Leptocerus sp.*	*Sympetrum fonscolombei**
*Bithynia tentaculata**	*Radix labiata**
Soft altered sites	*Dugesia* sp.*
Oligochaeta**	*Dreissena polymorpha**
*Pyrgula annulata**	*Acroloxus lacustris**
*Helobdella stagnalis**	*Asellus aquaticus**
	*Ancylus fluviatilis**

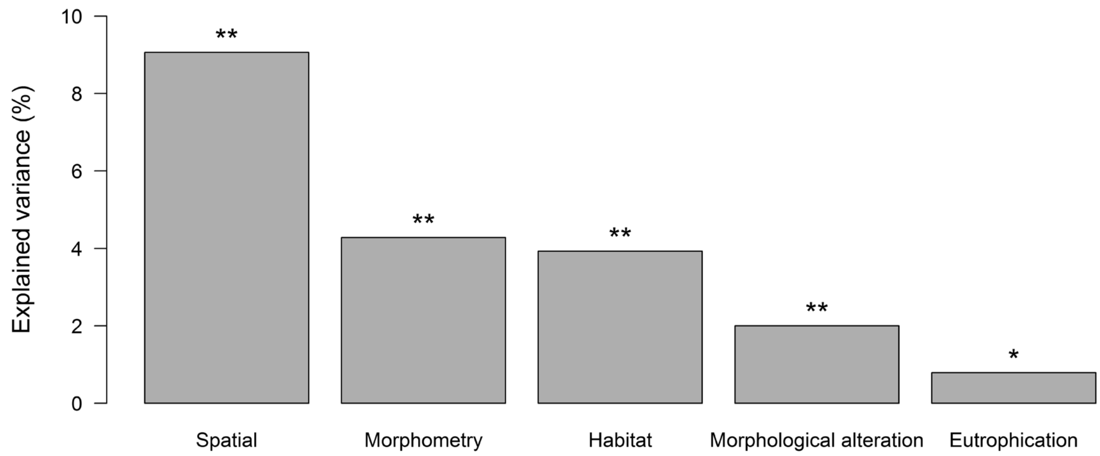

Figure 3. Results of the variance-partitioning analysis. The unique contribution of each explanatory variable group is reported as percentage of total variance in macroinvertebrate assemblage composition. The statistical significances of the fractions (after permutation tests) are: ** = $p < 0.01$, * = $p < 0.05$.

spatial scale) ranked among the most important factors structuring littoral invertebrate communities, in agreement with the findings of other studies (Tolonen et al. 2001, Brauns et al. 2007b, McGoff and Sandin 2012). Therefore, we can infer that the littoral community was structured by factors acting at several spatial scales, an inference consistent with the results of previous studies on benthic communities in the littoral zone of boreal lakes (Johnson and Goedkoop 2002) and on the sublittoral and profundal zones of subalpine lakes (Pilotto et al. 2012). Our results also support the multiscale filtering framework (Poff 1997). In this framework, the regional pool of taxa is subjected to constraints, or filters, which act at finer spatial scales through selective habitat forces to determine local macroinvertebrate community composition, as already has been demonstrated for invertebrates (Townsend et al. 2003, Johnson et al. 2007) and other freshwater biota in rivers (e.g., fish; Kwon et al. 2012).

The size of the lake significantly influenced the composition of the littoral community. On the contrary, the communities may be only secondarily influenced by the slope of the littoral zone, which may be important for determining the prevailing habitat type as suggested by Tolonen et al. (2001).

The habitat quality and complexity of the riparian and littoral zones (LHQA-total, LHQA-littoral, and LHQA-riparianscores) and the land use in the buffer zone within 200 m of the shoreline were among the most important variables defining the ordination gradients (PCA, Table 4). The LHQA-littoral score accounts for several features of the littoral zone including the diversity of sediment types, the diversity and extent of macrophyte cover, and the presence of shelters against fish predation (Rowan et al. 2006). Thus, habitat complexity seems to be a prominent factor structuring invertebrate communities (Cheruvelil et al. 2002, McGoff and Irvine 2009, Jurca et al. 2012). A high-quality riparian zone may strongly influence the benthic community by providing shade, roots, and woody debris (Brauns et al. 2007b), filtering terrestrial sediments and nutrients, and providing suitable habitats for survival and dispersal of invertebrates adult stages (Petersen et al. 2004). Shade and filtration provided by fringing vegetation also are important for unionids (Hastie et al. 2003, Österling and Högberg 2013).

Nutrient concentrations and phytoplankton biomass were the variables most strongly related to the PCA eutrophication gradients at the site and lake scales. Nutrient enrichment is the main trigger of the eutrophication process, and, in the initial stages, enhances primary production and leads to an increase of phytoplankton biomass (Rasmussen and Kalff 1987). However, eutrophication had a weaker effect on littoral communities than other gradients (Fig. 2).

Indicator taxa of environmental and spatial variables

Several taxa showed distinct preferences for particular microhabitats or environmental conditions. Oligochaeta were associated with sand habitats, and were the strongest indicator of soft altered sites. Several investigators have reported that Oligochaeta dominate lacustrine fine sediments in littoral and profundal zones (Wiederholm 1980, Sauter and Gude 1996, James et al. 1998, Donohue et al. 2009). *Dreissena polymorpha*, an invasive species found only in some lakes in northern Italy, uses stable solid substrates as attachment sites (Hecky et al. 2004, Orlova and Panov 2010), and it preferred morphologically altered sites. Similar habitat preferences were found for *Caenis macrura*. Gergs et al. (2011) hypothesized that *Caenis* spp. were linked to the availability of *D. polymorpha* biodeposits, which are used as food. *Echinogammarus veneris* was rarely found in samples from northern Italian lakes, whereas it was abundant in large, deep, oligotrophic lakes in central Italy (Bazzanti et al. 2012). This species was associated with increasing lake size, and it was an indicator taxon for unmodified shorelines. *Bithynia tentaculata* was negatively associated with soft substrates and positively associated with macrophytes and hard substrates. This association probably is related to its feeding behavior because this taxon is a grazer and feeds on periphyton (Cummins and Wilzbach 1985), which is strongly dependent on the presence of stable substrate. *Ischnura elegans* was characteristic of natural shorelines, in accordance with the findings by Brauns et al. (2007b), who suggested that higher abundances of *B. tentaculata* and *I. elegans* in natural littoral habitats are favored by the presence of fringing vegetation roots, which are particularly abundant there. Ceratopogonidae and *Dugesia* sp. were associated with eutrophic conditions, which is consistent with current knowledge (Rosenberg and Resh 1993, Gabriels et al. 2010).

Relative impacts of morphological alteration and eutrophication on community composition

Littoral benthic communities were more strongly associated with morphological alterations than with eutrophication, after accounting for spatial structure, and this result suggests a stronger impact of morphological alteration than of eutrophication on littoral benthic communities. On the other hand, the impact of eutrophication on benthic macroinvertebrates is strongest in the profundal zone (Bazzanti et al. 2012, Pilotto et al. 2012), but barely weaker than the impact of morphological alterations in the sublittoral zone (Pilotto et al. 2012). Thus, the effect of morphological alteration on macroinvertebrates seems to decrease from the littoral and sublittoral to the profundal zones, whereas the opposite trend occurs for eutrophication. Therefore, littoral invertebrates appear to be more sensitive indicators of morphological alterations than of eutrophication (McGoff and Sandin 2012).

Our study, like all observational studies, has limitations. For example, we cannot exclude the possibility that other (unknown) variables, such as toxic runoff or fish predation, could affect benthic communities. However, it is highly unlikely that unmeasured factors would affect the main result of our study, i.e., the relative impacts of morphological alteration and eutrophication, because their occurrence would eventually be represented by the fraction of spatially structured environmental variables (if acting at lake scale) or by the fraction of unexplained variance (if acting at site scale) resulting from the variance-partitioning analysis.

We suggest that evaluation of the effects of morphological pressures should be based on littoral macroinvertebrate assessment studies whereas methods based on profundal invertebrates should be preferred when the impact of eutrophication is the focus. Other groups of organisms respond to morphological alterations of the shoreline. For example, in a study in Minnesota lakes, macrophyte coverage was heavily reduced (66%) as a consequence of shoreline development (Radomski and Goeman 2001), and macrophyte-based assessment methods are currently being implemented in Europe to address morphological alterations of lakeshores (Birk et al. 2012). In contrast, the response of fish to morphological alterations is not clear. Jennings et al. (1999) showed that fish assemblages in littoral zones of 17 Wisconsin lakes were affected mainly by habitat complexity. Taxon richness was higher in complex habitats associated with natural shorelines and man-made rip-rap structures. Morphological alterations had negligible effects on fish assemblages in a study of 67 lakes in Germany (Mehner et al. 2005), but they significantly altered fish spatial aggregation in a study by Scheuerell and Schindler (2004). These contrasting results may be a result of the high mobility and diversity of feeding behaviors of fish, which can interact with different lake zones, from littoral to profundal (Schindler and Scheuerell 2002).

Conclusions

Methods for assessing lake ecological condition based on littoral benthic fauna should be used for assessment of the impacts of morphological alterations. In our study, once spatial variation was accounted for, the effect of morphological alterations to shorelines on littoral invertebrate communities was 2.5 times stronger than the effect of eutrophication. In addition, our results indicate that a biological assessment method based on littoral invertebrates in Italy should be regionally specific and should target morphological alterations once other sources of variation are ruled out. Although many bioassessment methods are converging toward Europe-wide (see Birk et al. 2012 for a recent review), we advocate that region-specific methods (for subalpine and volcanic lakes) might enhance the validity of results and improve the management of those water resources. In addition, the large within-lakes variance in our study seems relevant. Attempts to explain at least part of it with environmental variables measured at the appropriate scale (i.e., habitat) should be included in future studies to increase efficiency of the assessment.

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